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## EFFECT OF SOIL TEMPERATURE ON THE GROWTH OF BEAN PLANTS AND ON THEIR SUSCEPTI- BILITY TO A ROOT PARASITE

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The general opinion prevails that temperature plays an important rôle in the infection of a host by a fungous parasite. The experimental data showing just what this rôle is, however, are very meager. In the case of infection of aerial parts other factors are interrelated with temperature, such as persistence of moisture for spore germination, rapidity of germination of spores, and so forth, but in the case of infection of roots by organisms persisting in the soil these conditions ordinarily do not enter. Apparently the soil-inhabiting parasites are largely capable of saprophytic existence so that, given the requisite amount of soil moisture to maintain plant development, the parasite is able to grow and reach the roots of a susceptible host. Gilman<sup>1</sup> has recorded observations on the relation of infection by *Fusarium conglutinans* Wr. on cabbage to soil temperature conditions and thinks a high soil temperature favorable to infection. Gilman<sup>1</sup> continued this work with *F. conglutinans* and appears to have established the point just mentioned, although the control of conditions in some of his experiments was not all that might be wished for. Tisdale<sup>2</sup> arrives at similar conclusions in connection with the infection of flax (*Linum usitatissimum*) by *Fusarium Lini* Bolley and states that the low critical temperature is about 15°-16° C.

<sup>1</sup> Gilman, J. C. The relation of temperature to the infection of cabbage by *Fusarium conglutinans* Wollenw. (Abstract.) *Phytopathology* 4: 404. 1914. Cabbage yellows and the relation of temperature to its occurrence. *Ann. Mo. Bot. Gard.* 3: 25-82. 1916.

<sup>2</sup> Tisdale, W. H. Relation of temperature to the growth and infecting power of *Fusarium Lini*. *Phytopathology* 7: 356-360. 1917.

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The writer undertook an investigation of somewhat similar nature, using the bean, *Phaseolus vulgaris*, as host and *Fusarium martii phaseoli*<sup>3</sup> for the parasite, a fungus that has been shown by Burkholder<sup>3</sup> to be the cause of a serious disease of beans in New York.

The work was performed in the laboratory of plant physiology of the Johns Hopkins University, Baltimore, Maryland, where the writer was fellow by courtesy during the academic year 1916-17. His thanks are due the authorities of that institution for the facilities afforded him and special acknowledgment is made of the critical advice given him by Prof. B. E. Livingston, under whose immediate guidance the work was done. While the investigations are by no means completed, some of the physiological features of the results thus far obtained are of sufficient interest to warrant this note.

The plants were grown in cylindrical vessels of tinned sheet-iron, 17 cm. tall and 15 cm. in diameter, which in turn were placed in a water bath. The garden soil used was first heated in an autoclave for one hour at a temperature of 110° C. and it was then made uniform by repeated sifting. The culture vessels were filled and nearly uniform packing was obtained by letting the soil fall into place always from the same height. Water was supplied by means of the Livingston auto-irrigator,<sup>4</sup> two cylindrical porous clay cups being used, each with an exposure to the surrounding soil of approximately 121 square centimeters.

The irrigation water was drawn directly from the water of the bath and care was taken to have the supply uniform, so as to avoid difference in soil moisture content that might influence the growth of the plants.

Since the water level was nearly as high outside the cylinders as was the level of the soil within, it was necessary, while the plants were

<sup>3</sup> Burkholder, W. H. Some root diseases of the bean. (Abstract.) *Phytopathology* 6: 104. 1916. Bean diseases in New York State in 1916. (Abstract.) *Phytopathology* 7: 61. 1917.

Burkholder states that *Fusarium martii* Ap. & Wr. does not produce infection on the bean but that the fungus from bean is nearly identical with this species. The name *martii phaseoli* has not been used previously and is only introduced here as a matter of convenience.

<sup>4</sup> Livingston, B. E. A method for controlling plant moisture. *Plant World* 11: 39-40. 1908.

Hawkins, Lon A. The porous clay cup for automatic watering of plants. *Plant World* 13: 220-227. 1910.

Livingston, B. E., and Hawkins, Lon A. The water-relation between plant and soil. *Carnegie Inst. Wash. Publ.* 204: 3-48. 1915.

small, to retard the flow of water into the cups. This was accomplished by introducing mercury columns of equal heights into all the supply tubes. Later the mercury was not needed and was removed.

The water baths employed were three in number, each 60 cm. in diameter and 25 cm. deep (ordinary galvanized iron laundry tubs) thus giving space for seven culture vessels each. A wood grating at the bottom supported the culture vessels and allowed them to be submerged to within one centimeter of the top.

Three temperatures,  $34^{\circ}$ ,  $22^{\circ}$ , and  $15^{\circ}$  C., were arbitrarily decided upon, but this choice was governed somewhat by the facilities available. The highest temperature was obtained by means of an electric heater under thermostatic control, and was maintained uniformly throughout the course of the experiment.

The medium temperature followed that of the culture room; there was no special control in this bath. Because of the great bulk of water, the fluctuation in temperature was not very great. The range was from  $20^{\circ}$  to  $23^{\circ}$  C. (usually  $21^{\circ}$  to  $22^{\circ}$ ) whereas the diurnal variation in the temperature of the greenhouse room was large,  $12^{\circ}$  to  $28^{\circ}$  C. In our present state of knowledge of the influence of soil temperature on host or parasite this fluctuation is to be regarded as of little consequence but obviously some constant temperature might have been maintained with very little difficulty, by employing such an outfit as was used for the highest temperature.

A constant water level was maintained in each of the two warmer baths by means of a Mariotte flask.

The lowest temperature was obtained by passing a continuous stream of tap water through the bath. When the water flowed at the rate of 1,500 cc. per minute a temperature of  $15^{\circ}$  was maintained, during the winter months. On very warm days a rise of two or three degrees sometimes occurred. The total range was from  $14^{\circ}$  to  $18^{\circ}$ .

The surface of the water in the baths was covered with a thick paraffin oil to reduce loss of heat by evaporation and to eliminate the vapor blanket that would otherwise have been present over such an exposed body of water. Later it was found that a covering of ordinary paraffin (melting point about  $50^{\circ}$ ) was very much better for the purpose. This was melted and poured on the water, where it was allowed to spread and harden.

The soil in four of the culture vessels of each series was contaminated by sprinkling in it, when nearly full, some soil heavily laden with

viable spores and mycelium of *Fusarium martii phaseoli* from culture. The fungus, which was supplied through the courtesy of Dr. W. H. Burkholder, had been maintained for several months in pure culture but the medium (bean-pod decoction agar) was uniform throughout the period, and a sub-culture had been made every ten days.

Pure-line seeds of a pea bean,<sup>5</sup> were disinfected externally with a 1 to 1,000 solution of mercuric chlorid, after which they were sprouted in a moist chamber. They were planted on January 10, 1917, six<sup>6</sup> seeds in each culture.

When the cotyledons had broken through the ground all plants were inoculated with *B. radiculicola* by injecting into the soil about the roots one cubic centimeter of a heavily laden water suspension of this organism taken from bean nodules, and the number of plants per pot was reduced to four.

After twelve days the plants with soil temperature at 34° were developing the first trifoliate leaf; those at 22° had just spread the first pair of true leaves and those at 15° were not all through the soil surface. On the forty-fifth day the plants at 34° were beginning to blossom while those at 22° began blossoming eleven days later. The plants at 15° were either dead or very poor and none developed satisfactorily. A single one of these cold-soil plants finally reached a height of about 15 cm. and produced one blossom but did not set a pod. It is to be borne in mind that the air temperature here was practically the same as that of the plants with soil temperature of 22°.

Unfortunately some of the plants in the control cultures became infected, the contamination apparently being carried by numerous small insects that were abundant on the plants. In the cultures at 22° five of the twelve plants were diseased and in those at 34° eight of the twelve plants were affected. All of these plants were affected relatively late as compared with the inoculated plants, so that it is impossible to judge what amount of damage may be attributed to the

<sup>5</sup> The seed was supplied through the courtesy of the Department of Plant Breeding, Cornell University and is maintained under the department number 1986-2.

<sup>6</sup> From the outcome of this experiment and numerous others subsequently performed with beans of this and another pure line, and with beans secured on the open market, it is very evident that not enough seeds were used at the outset. After seeds of uniform size and appearance are selected it is safe to allow for only about 25 percent as likely to yield plants entirely free from defect and of perfectly uniform appearance. Weak plants frequently cannot be detected for ten days or two weeks after the plants emerge from the soil.

disease, but it was obvious at harvest time that the plants in two of the control cultures of the series of 24° were severely injured.

In addition there were "weak" plants in nearly every culture. These could not be detected as such for two weeks or more after the plants were up and it was then too late to correct for the trouble. In fact it was thought for some time that some of these plants were ones on which infection had been particularly severe. As there were plants of varying degrees of "weakness" it is not possible to throw the poor plants out of consideration.

Furthermore, it is not possible to make a comparison between the cultures grown at the two temperatures because of the fact that the plants grown at high temperature developed more rapidly from the very beginning and thus matured under a different set of air conditions. In this experiment this meant that the plants grown at the highest temperature had very much less sunshine than those grown at 22°. The difference is noticeable in part in the total dry weight of seed, but some of the difference is attributable to a more severe infection on control plants grown at the high temperature.

Finally the difference in growth at the two different temperatures might have been due in part to a difference in air temperature. Thermometers suspended over the water baths at a distance of 15 cm. from the surface showed constantly a higher temperature over the bath at 34° than over the ones at 22° and 15°. The difference varied from .5° to 4.5° and averaged from 3° to 4° higher.

With these four considerations in mind it may now be stated that the average yield per plant for "healthy" plants in the series at 34° was 1.451 grams of air-dry seed. For the infected plants the average was 1.081 grams. Thus the presence of this *Fusarium* on the roots of beans under the conditions stated resulted in a direct loss of 25.5 percent. For the cultures grown with a soil temperature of 22° the average yield per healthy plant was 2.361 grams. For the inoculated plants it was 1.557 grams. Here the reduction in yield on account of disease was 34 percent.

The most interesting feature of the experiment is the fact that these beans grew faster and matured a crop earlier with the higher soil temperature. The relatively small difference in air temperature may account for some of this difference in growth but certainly cannot entirely account for the results obtained. Wholly aside from its scientific interest the question may have an important practical bearing for

those engaged in the production of flowers and vegetables under glass, and from either standpoint is worthy of further attention.

The idea of supplying bottom heat has been used extensively by florists<sup>7</sup> for starting cuttings, but not for growing crops. Plant physiologists do not seem to have studied the problem, judging by the absence of literature on the subject, but this experiment with beans and some trials with radish (*Raphanus sativus*), cucumber (*Cucumis sativus*), and tomato (*Lycopersicum esculentum*) indicate that root temperature and foliage temperature are readily separable as conditions influencing the growth of plants.

With respect to susceptibility due to environmental changes it would seem that in the case of temperature as applied in this experiment the relation between host and parasite cannot be analyzed readily. The experiments show that the host is influenced markedly by a change in soil temperature so that it is impossible to make a direct comparison of various temperature conditions because of the slow action of the parasite. If the parasite made a rapid invasion and killed the host outright within a few days there would be an opportunity to grow all plants under identical conditions until the day of inoculation but even then the sudden change of soil temperature might have an even more marked effect on the physiological condition of the host, perhaps changing its susceptibility in a very pronounced manner. In the case of this disease, and of the majority of root diseases, prompt death of the host does not follow because some water continues to enter even after the roots have been killed and especially because on most plants new roots generally push forth above the point of infection.

It will be necessary to study under controlled conditions the behavior of the uninfected host when subjected to certain changes in this one environmental condition, and that of the parasite in the same way, in order to determine the true relation of host and parasite. This involves the control of all the known conditions affecting the growth of plants, including light, a method for doing which has only been hinted at<sup>8</sup> to date.

The physiology of the fungus here used has not been the subject of investigation as yet, but in some preliminary experiments on the rate of growth of the fungus at different temperatures it was found that the diameters of the thalli on bean broth agar in petri dishes varied

<sup>7</sup> White, E. A. The principles of floriculture. p. 162-164. New York. 1915.

<sup>8</sup> Livingston, B. E. Plant World 20: 11. 1917.

with the temperature. In one instance, at the end of five days, the diameters of the thalli in millimeters for the stated temperatures are shown in the accompanying table.

TABLE I

*Diameters in Millimeters of Thalli of Fusarium martii phaseoli when Grown for Five Days at the Temperatures Indicated*

Temperature °C.	Diameter of Thallus Millimeters
12-13 .....	8
15-16 .....	12
17-18 .....	15
19-21 .....	17
23-25 .....	23
26.5-27.5 .....	28
30.5-31.5 .....	34
35-35.5 .....	13
38.5-39 .....	No growth

It appears from the table that the highest temperature selected for the experiment was one near, but perhaps slightly above, the optimum for the growth of this fungus, but it is to be noted that growth takes place at a temperature much below the lowest temperature selected and infection occurred on inoculated plants in the cultures at all three soil temperatures employed.

It is unfortunate that a low temperature was not selected that would at least have permitted the growth of beans even though poorly. It is well known<sup>9</sup> that beans require a warm soil for their best development. In a cold soil presumably bean plants would not have as great vitality and might have proved particularly susceptible to this hemiparasite. Likewise, in the case of cabbage it is well known that the plants do well in a cool summer and poorly in a warm one. At the higher temperatures the plants may possess a lower degree of vitality and hence should be more susceptible to facultative parasites. This point Gilman passes over lightly in his work.

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<sup>9</sup> Reynolds, J. B. Temperature in relation to seed. Ont. Agr. Col. Rept. 29 (1903): 9-11. 1904.

Sevey, Glenn W. Bean Culture, p. 7. New York. 1914.